Assisted Reproduction Techniques for avoiding inherited diseases

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showing we care

Guy's and St Thomas' NHS
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1. Flinter

What are the circumstances in which parents could not use alternative reproductive technologies to avoid passing on a genetic disease?
What is the chance of PGD being successful for specific genetic conditions?

2. Braude

What is the rate of successful pregnancies using these technologies and what factors affect this rate?
What room for improvement is there in these technologies?

3. Racowsky

How does regulation and availability of alternatives technologies vary and compare internationally?
What are the ramifications of these variations on practices in private clinics?
Embryo selection for desired traits and exclusion of polygenic disease risk

Definitions:

Pre-implantation Genetic Diagnosis (PGD)

WHO: Pre-implantation Genetic Testing (PGT)

PGT-M for monogenic (single gene) disorders

PGT-SR for structural chromosome rearrangements

PGT-A for aneuploidy screening (previously PGS)

Reproductive options for couples with genetic disorders

- Reproductive roulette
- Prenatal diagnosis/TOP
- Gamete donation
- Adoption
- Remain childless
- PGD

Who uses PGD?

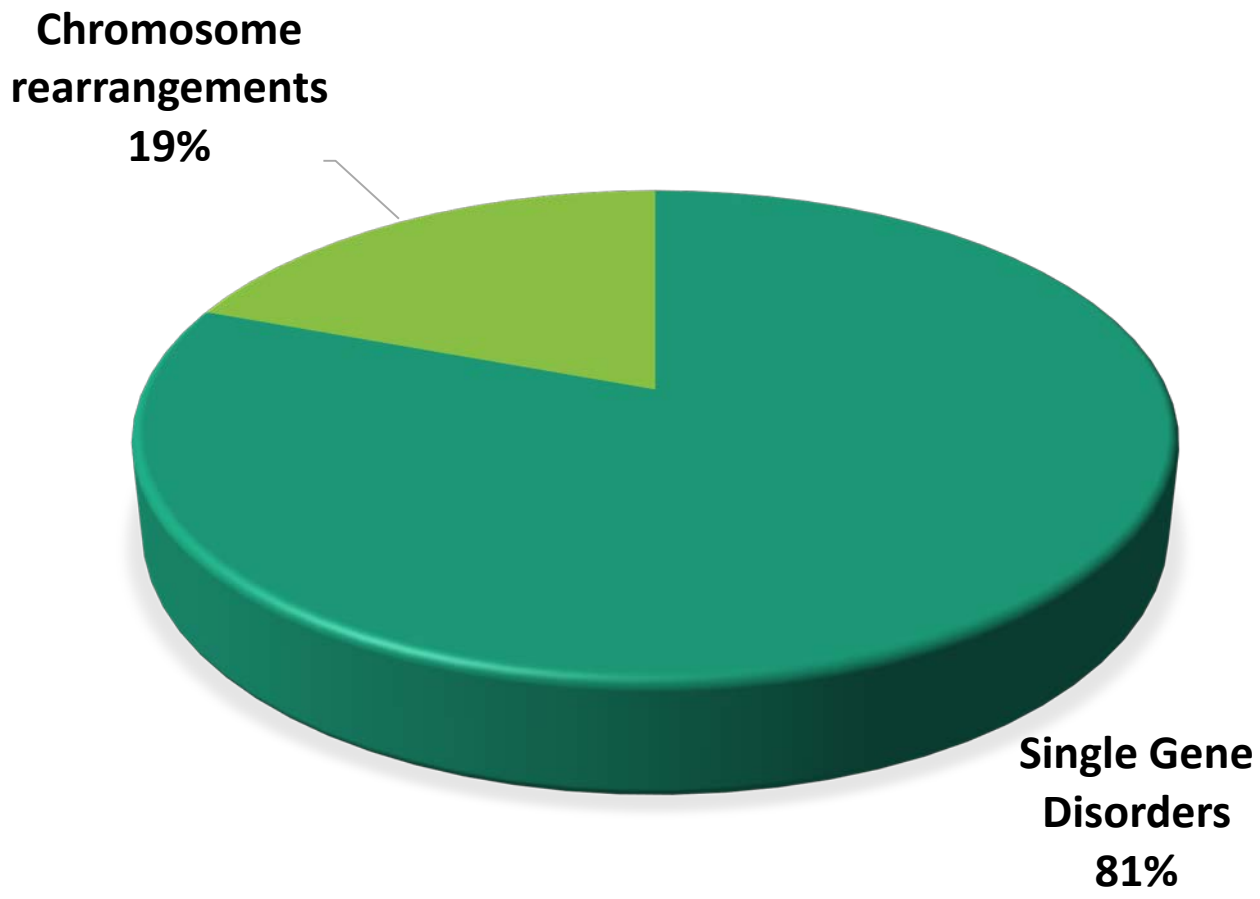
People at **significant** risk of having a child with a **serious** genetic disorder who would **not** consider prenatal diagnosis and termination of pregnancy

i.e. a **very small minority** of those who carry a genetic condition

How culturally acceptable is PGD?

- Decisions are couple-specific
- Discarding affected embryos
- Failure to use unaffected embryos

PGD at Guy's: 435 cycles in 2018



What is the chance of PGD working?

- 1 in 3 chance of live birth if get to embryo biopsy
- 1 in 2 chance of live birth if have an embryo replaced

When is PGD most likely to work?

- Young people
- Normal fertility
- Normal female BMI
- Non smokers
- Previous successful conception
- Healthy female partner

When is PGD least likely to work?

- Multiple genetic conditions
- Low number of embryos to test

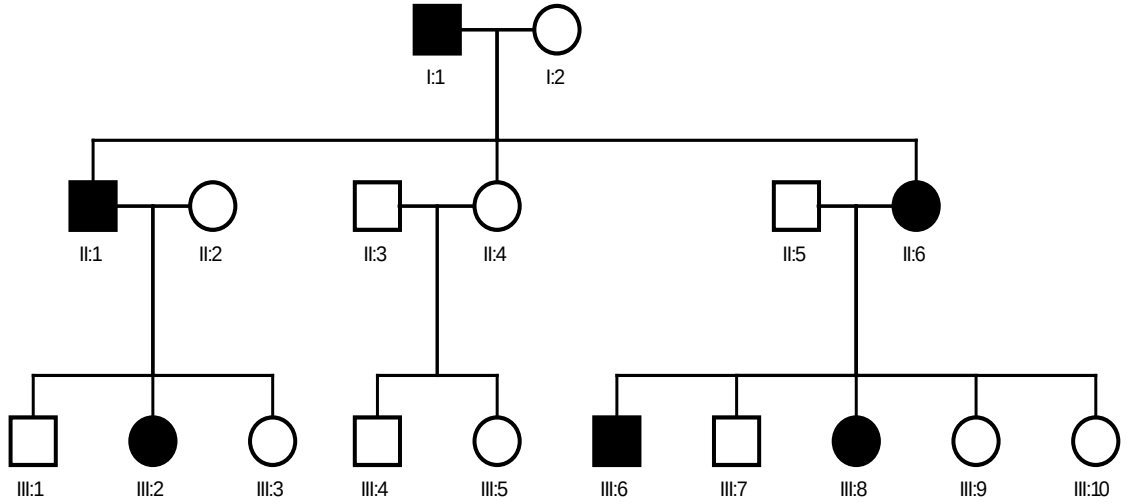
Other challenges

- Poor maternal health: use surrogate
- No uterus: use surrogate
- Single women: use sperm donor
- Same sex couple: use sperm donor

- Pre-existing sick child
- Need for an HLA match

Autosomal dominant inheritance e.g. AD polycystic kidney disease

- Males & females affected
- Male to male transmission
- 50% risk to offspring
- New mutations occur
- Reduced penetrance
- Variable expressivity



When is there no chance of finding an unaffected embryo? (AD)

One parent homozygous for an AD condition

e.g. familial hypercholesterolaemia
Huntington's disease

(many homozygous AD conditions are lethal)

More common challenges with AD inheritance

Few unaffected embryos - 50% affected

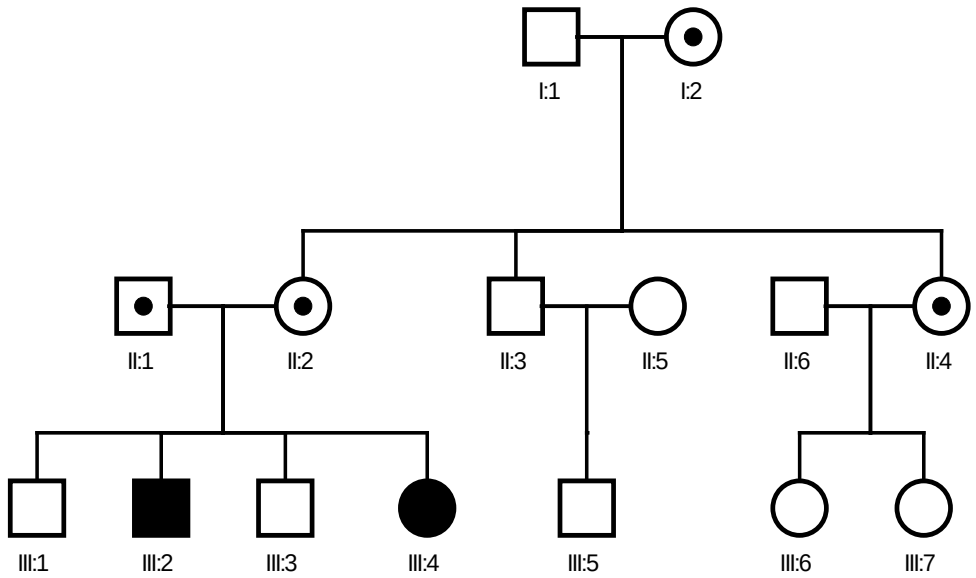
Multiple diseases: e.g. Marfan's (MFS) and BRACA:

If one parent has MFS and the other carries a BRACA mutation:

1/4 MFS, 1/4 BRACA, 1/4 MFS and BRACA, **1/4 unaffected**

Autosomal recessive inheritance

- Both parents gene carriers
- Affected individuals in one generation
- Consanguinity may be present
- Recurrence risk 1 in 4
- 2/3 risk that healthy sib is a carrier
- eg ARPKD



When is there no chance of finding an unaffected embryo? (AR)

Both parents have the same AR condition: all gametes carriers, all embryos affected

e.g. non-syndromic sensorineural deafness

sickle cell disease

thalassaemia

More common challenges with AR inheritance

- **Two recessive conditions (consanguinity)**

  9/16 chance of finding unaffected embryo

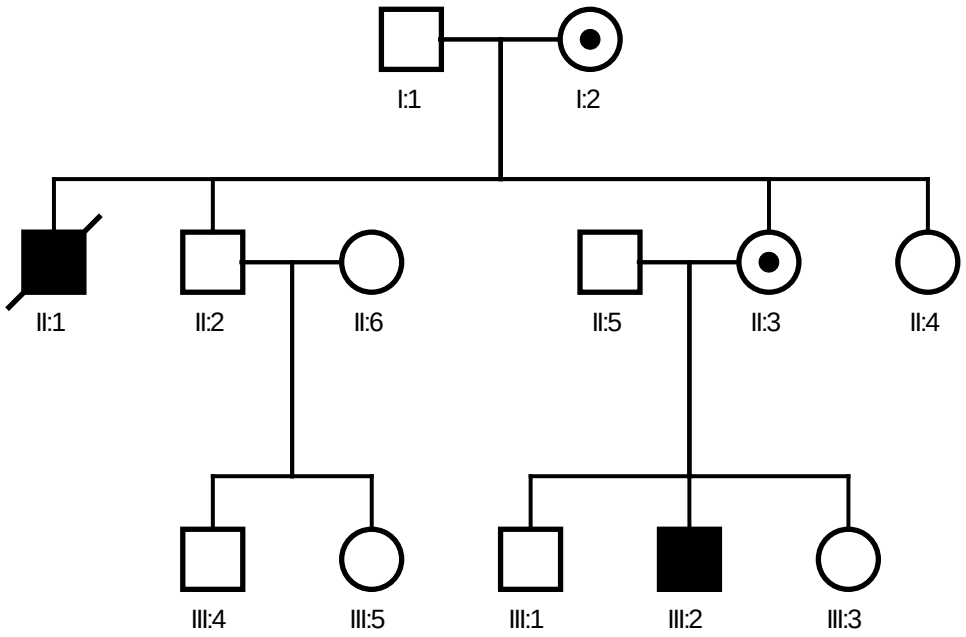
- **Exclusion of AR disease plus HLA matching**

  3/4 embryos unaffected with the disease
  1/4 HLA match

  Chance of unaffected embryo with HLA match = ¾ x ¼ = 3/16

X-linked recessive inheritance

- Females only affected rarely
- No male-male transmission
- Daughters of affected males will be carriers
- 1 in 2 sons of carrier females will be affected
- eg Alport's syndrome



More common challenges with XL inheritance

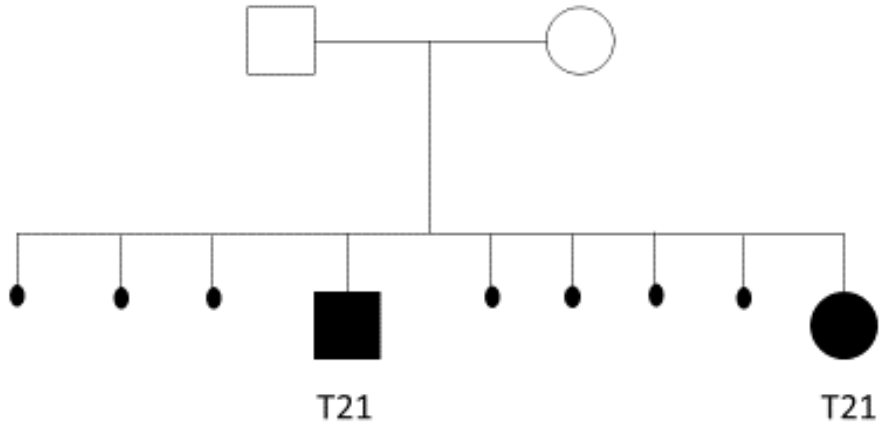
- What to do about carriers?
- Phenotype in female carriers may be unpredictable

When is there no chance of finding an unaffected embryo? (chromosomal)

- One parent carries a homologous Robertsonian translocation

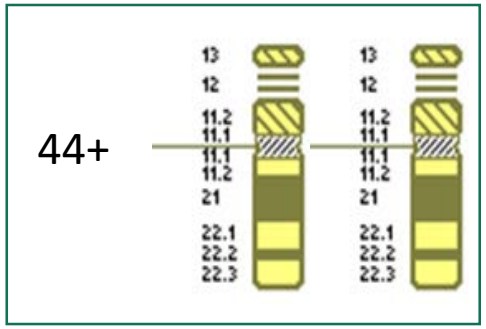
- All gametes will have two copies of the same chromosome, so after fertilisation there will be three (e.g. trisomy 21)

Pedigree of a homologous Robertsonian 21;21 translocation carrier

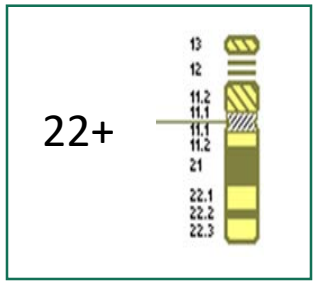


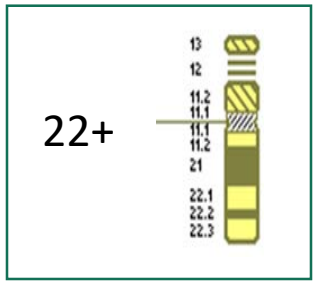
Homologous Robertsonian (or iso-) chromosomes

Normal complement



gametes





sperm

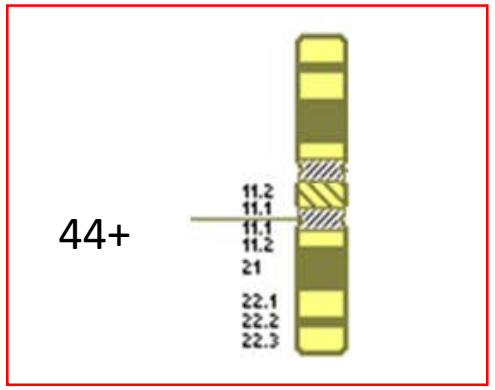
23

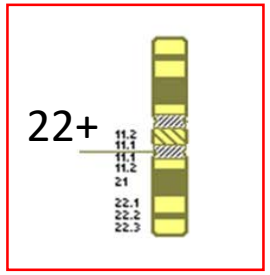
embryos

46 - normal

46 - normal

der(21;21)(q10;q10) carrier





22

46,der(21;21)
Down
syndrome

45,-21
Monosomy
21 - lethal

Carriers of homologous Robertsonian translocations

- zero chance of normal offspring*
- PGD is ineffective, as there will be no normal embryos to select.

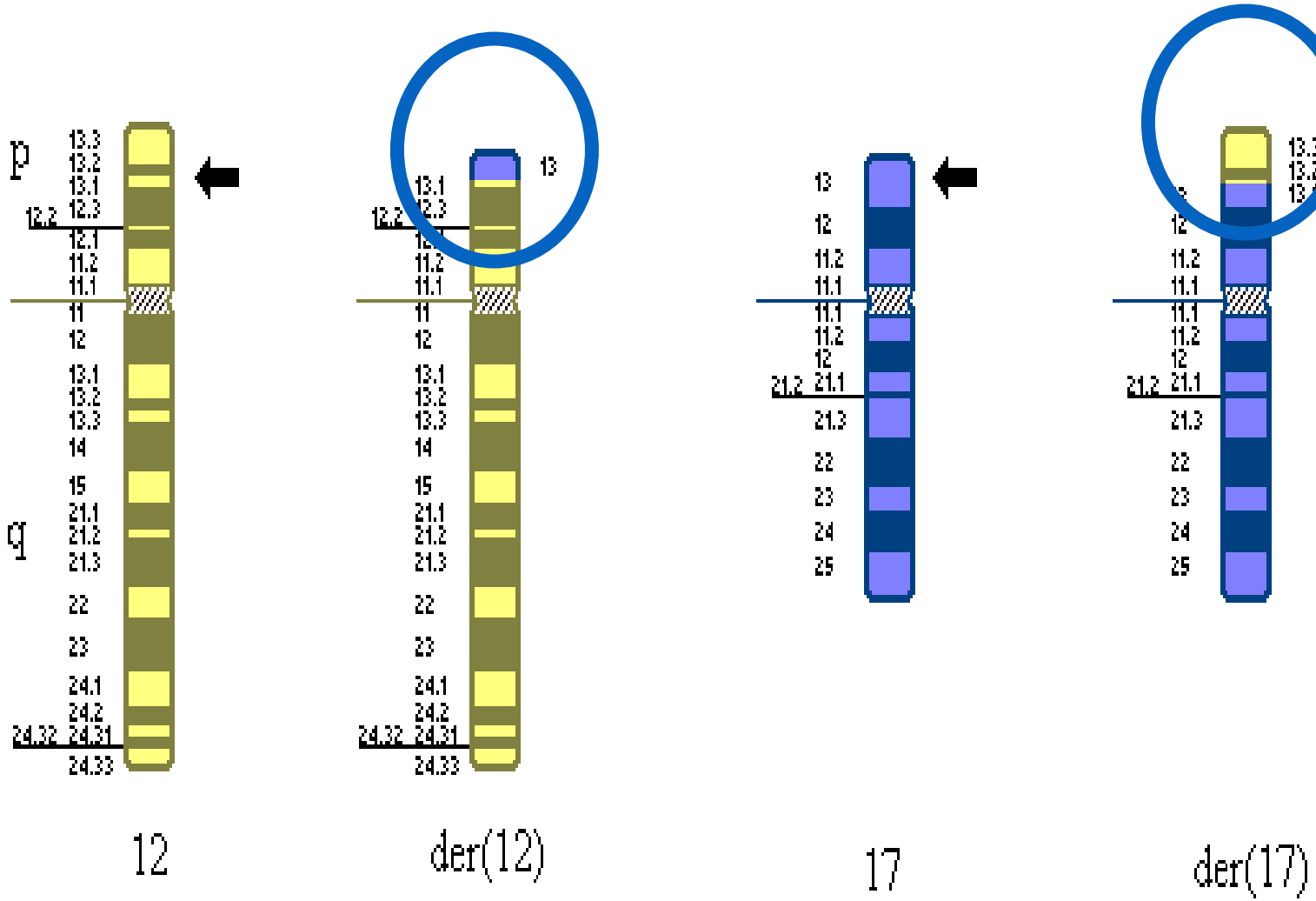
**Extremely rare cases of trisomy or monosomy rescue have been reported, but where the chromosome involved is 14 or 15, rescue will result in UPD, with phenotypic consequences.*

- Germline genome editing not possible – carriers need to use donor gametes

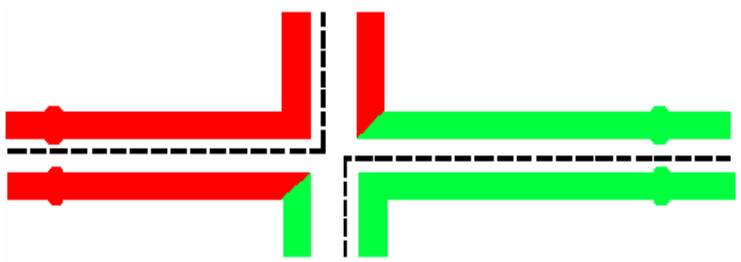
More common problems with chromosome rearrangements….

- Numerous different rearrangements possible: 1/500 carriers.
- The outcomes can involve large imbalances which cannot be predicted and are likely to be different in each embryo
- PGD works for some couples (but germline genome editing would not be possible as there can be so many variants)

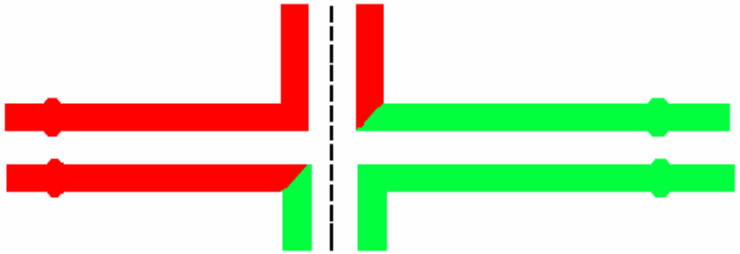
Reciprocal translocation: 46,XX,t(12;17)(p13;p13)



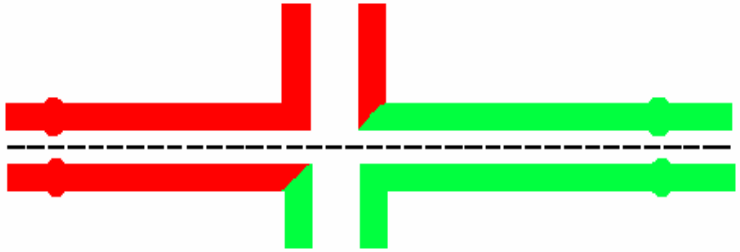
At meiosis, chromosomes pair along homologous regions to form “quadrivalents”, which can segregate to daughter cells in four different ways (segregation modes). Brackets show the empiric frequency of each mode.



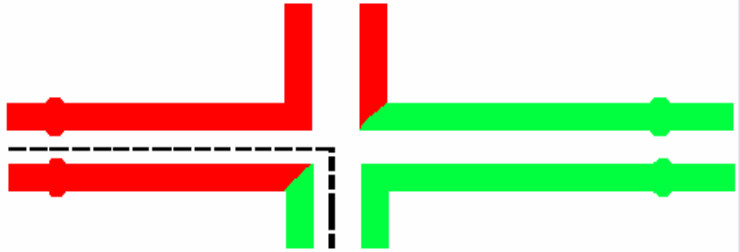
Alternate (46%)



Adjacent-2 (12%)



Adjacent-1 (37%)



3:1 (5%)

Reciprocal translocations

These 4 meiotic segregation modes result in 32 *different* genetic outcomes in gametes. Only 2 of these outcomes will give rise to a normal pregnancy.

In a cohort of embryos, each embryo may carry any one of these 32 outcomes.

An embryo with an abnormal chromosome complement will have genetic imbalance, usually involving two different chromosomes, and these imbalances will be many Mb in size.

PGD can be used to select embryos without genetic imbalance

When is PGD technically impossible?

Very rarely!

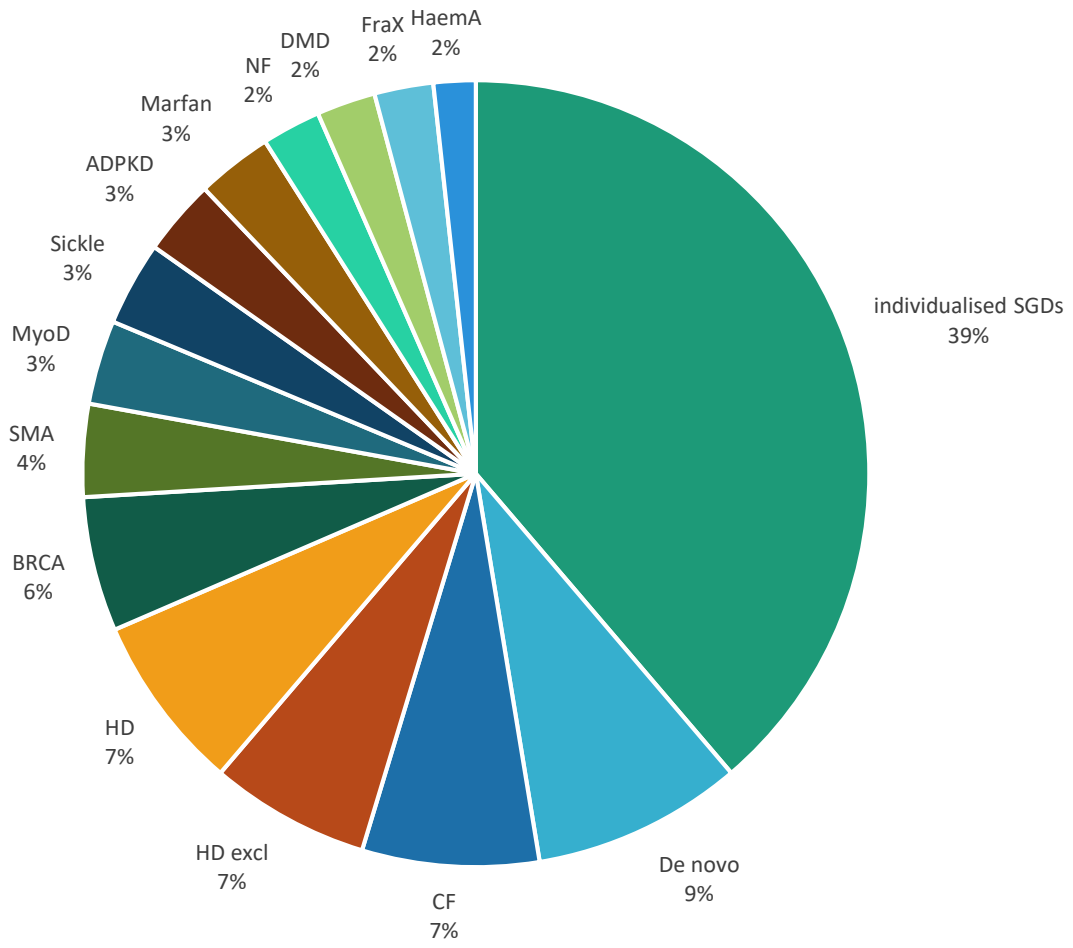
Good labs insist on 2 independent tests to score an embryo.

For Mendelian disorders either

- mutation plus a linked marker or
- two linked, flanking markers

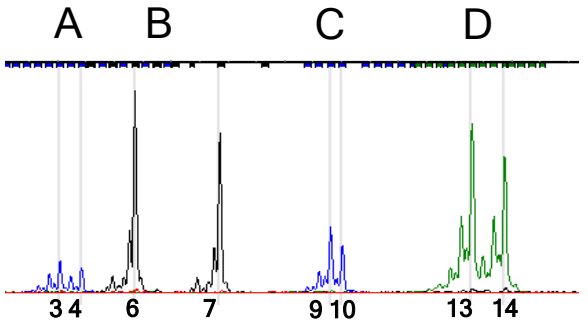
ESHRE guidelines <2% risk (good labs <1%)

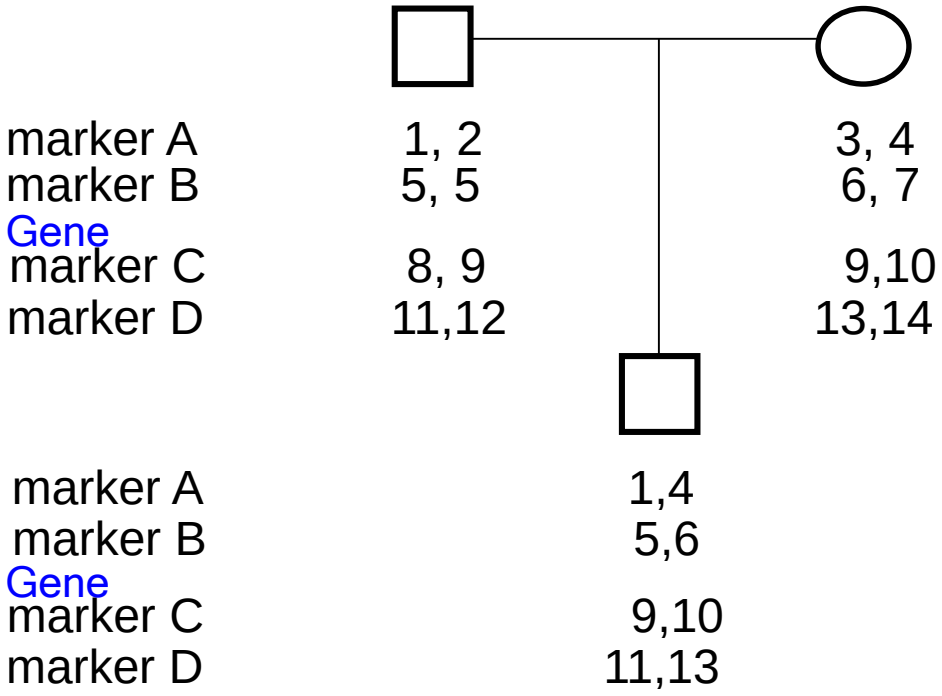
Range of Single Gene Disorders: Guy's 2018



Principle of Haplotyping (1)

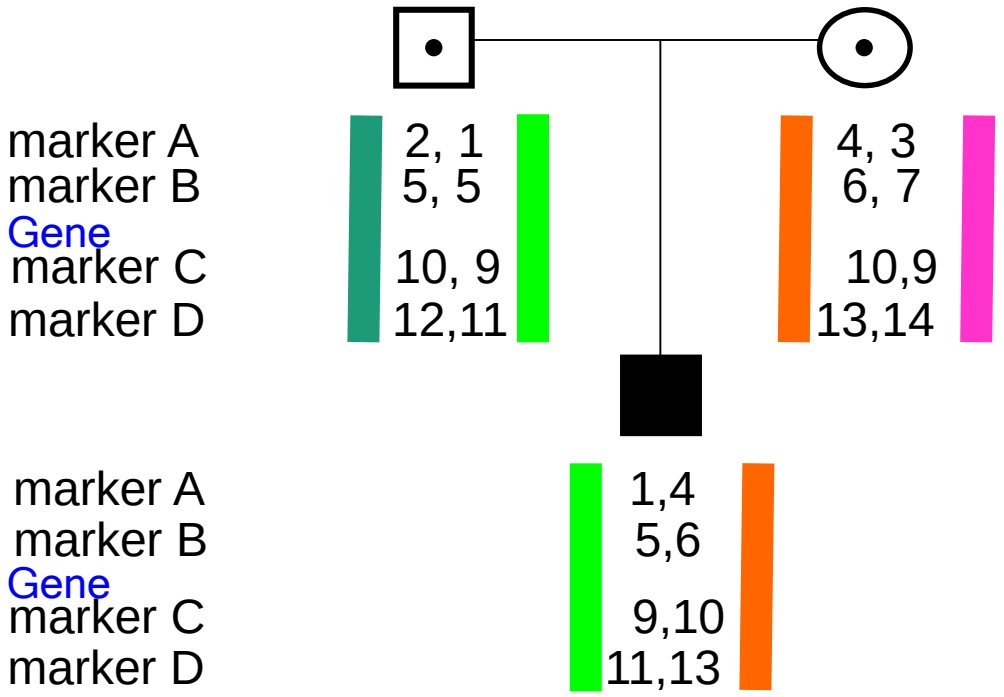
- **Chromosomes have different repeat lengths (alleles) at STR regions (markers)**
- **Obtain allele sizes of STR markers flanking the gene.**





Principle of haplotyping (2)

Assign phase of alleles at markers according to affected family member to create haplotypes (alleles inherited together on a chromosome)



The value of linkage

- Do not need to develop a test for the specific mutation
- Reveals any maternal cell contamination
- Reveals allele drop out
- Reveals cross overs
- **Reduces the risk of misdiagnosis**

Tricky situations:

De novo dominant mutation in affected parent:

- Cannot use linkage
- Need to develop a tailor made test for the specific mutation

PGD not possible: de novo FSH

FSH = fascio-scapulo-humeral muscular dystrophy

- Telomeric gene so only 1 linked marker if familial
- Big retraction 35kB to 11kB in gene plus homologous regions in chromosome 10
- De novo cases mean linkage not possible

(GGE not possible either)

Tricky situations: class 3 VUS

Variants of Uncertain Significance

- We cannot be sure they are the cause of the disease
- Not used for PGD unless there is other evidence to confirm that the variant segregates with the disease and is definitely in the right gene

Even in this situation, GGE to correct a VUS would not be acceptable. Must ensure that mutations corrected are pathogenic

When might GGE be helpful?

1. In some very rare situations when there is no chance of producing normal gametes

2. For couples who are unlucky and repeatedly produce no unaffected embryos (it's a numbers game)

But for most there are other options…….

Reproductive options for couples with genetic disorders

- Reproductive roulette
- Prenatal diagnosis/TOP
- Gamete donation
- Adoption
- Remain childless
- PGD

Thank you